

DEPARTMENT OF  
INDUSTRIAL CHEMISTRY

on a Hitachi 7170 Type automatic analyzer. The operational method was as follows. First, to 3  $\mu$ L of each specimen was added with 210  $\mu$ L of reagent A-1, and kept in a constant temperature at 37°C for 5 minutes. At this point of time, absorbance 1 was measured at a 5 main wavelength of 340 nm and a side wavelength of 570 nm, respectively. Furthermore, 70  $\mu$ L of reagent A-2 was added, and kept in a constant temperature at 37°C for 5 minutes. At this point of time, absorbance 10 2 was measured at a main wavelength of 340 nm and a side wavelength of 570 nm, respectively. A difference between the absorbances 1 and 2 was obtained and the value of each specimen was converted 15 using a control, whose HDL-cholesterol concentration was already known, as a standard solution. Reagents B-1 and B-2 were used in the same manner as above. The values by the control method were obtained in accordance with the Friedewald equation. The HDL 20 cholesterol values were obtained using PG pole produced by International Reagents Co., Ltd. The total cholesterol values were obtained using T-CHO reagent A that was produced by International Reagents Co., Ltd. The TG values were obtained using TG reagent A that was produced by International Reagents Co., Ltd. The results obtained are shown in Table 2. The method of the example gave satisfactory results as compared with the control method.

Reagent A-1

|                                 |            |
|---------------------------------|------------|
| Buffer solution                 | pH 7.8     |
| 25 Hydrazinium dichloride       | 100 mmol/L |
| Cholesterol dehydrogenase (CDH) | 20.0 U/mL  |

β-NAD 6.0 mmol/L  
LPL (derived from Chromobacterium viscosum) 6.0 U/mL  
Nonion K-230 (HLB value 17.3) 0.15%  
5 Sodium cholate 0.1%

Reagent A-2

Buffer solution pH 8.5  
CE (derived from Pseudomonas) 3.0 U/mL  
10 Nonion A-10R 0.5%  
Sodium deoxycholate 8.0 mmol/L

Reagent B-1

Buffer solution pH 7.8  
15 Hydrazinium dichloride 100 mmol/L  
β-NAD 5.0 mmol/L  
Cholesterol oxidase (COD) 0.3 U/mL  
LPL (derived from Chromobacterium viscosum) 6.0 U/mL  
20 Nonion K-230 (HLB value 17.3) 0.15%  
Sodium cholate 0.1%

Reagent B-2

Buffer solution pH 8.5  
25 Cholesterol dehydrogenase (CDH) 20.0 U/mL  
CE (derived from Pseudomonas) 3.0 U/mL

Nonion A-10R 0.5%  
 Sodium deoxycholate 8.0 mmol/L

Table 2

Unit: mg/dL

| Specimen                        | Control Method | Reagent A | Reagent B |
|---------------------------------|----------------|-----------|-----------|
| 1                               | 151            | 155       | 147       |
| 2                               | 173            | 188       | 168       |
| 3                               | 236            | 234       | 220       |
| 4                               | 79             | 79        | 66        |
| 8                               | 173            | 167       | 157       |
| 9                               | 170            | 173       | 163       |
| 7                               | 118            | 123       | 111       |
| 8                               | 87             | 93        | 81        |
| 9                               | 92             | 95        | 90        |
| 10                              | 64             | 72        | 64        |
| Correlation                     |                | 0.995     | 0.996     |
| Inclination of regression curve |                | 0.973     | 0.943     |
| Intercept of regression curve   |                | 7.235     | 0.083     |

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### Example 3

#### Experiment Examples

The following reagents were prepared and the effects of each factor were studied in Experiments 1 to 4. As specimens, HDL, LDL 10 and VLDL fractions were used, which were obtained by pooling serum 10, sampled from ordinary person, and followed by ultracentrifuge. The assays were practiced on a Hitachi 7170 Type automatic analyzer. The operation method was as follows. First, to 5  $\mu$ L of each specimen was added with 180  $\mu$ L of reagent 1-D, -E, -F, or -G, and kept in 15 a constant temperature at 37°C for 5 minutes. At this point of time, absorbance 1 was measured at a main wavelength of 340 nm and a side